

EXHIBIT B16

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Talc induces a pro-oxidant state in normal and ovarian cancer cells through gene point mutations in key redox enzymes

Objectives: Genital use of talcum powder is associated with increased ovarian cancer risk. Recent data from our laboratory suggests talc induces inflammation and pro-oxidant state in normal and ovarian cancer cells. We have previously reported that alterations in key pro-oxidant and antioxidant enzymes lead to a persistent pro-oxidant state in epithelial ovarian cancer (EOC) cells that is associated with specific single nucleotide polymorphisms (SNPs) in these enzymes. Here, we sought to determine whether talc enhances the pro-oxidant state in normal and ovarian cancer cells through the induction of point mutations corresponding to known SNPs in the key redox enzymes.

Methods: Normal ovarian, human epithelial ovarian cells (HOSEpic), normal fallopian tube (FT33), and EOC (A2780, SKOV-3, TOV112D) cell lines were treated with talc (100 µg/mL) for 48 hours. TaqMan® Genotype analysis utilizing the QuantStudio12K Flex was used to assess single nucleotide polymorphisms (SNPs) in genes corresponding to target enzymes: catalase (CAT), inducible nitric oxide synthase (NOS), superoxide dismutase (SOD3), glutathione peroxidase (GPX1), and glutathionereductase (GSR). ELISA was used to measure activities/levels of these key redox enzymes with point mutations in response to talc treatment. Data was analyzed with one-way ANOVA followed by Tukey's post hoc tests with Bonferroni correction.

Results: Of the enzymes tested, we identified an induction of specific mutations in only CAT, NOS, and GPX1 that correlated with alterations of their activities in talc treated cells as compared to their controls (see Table 1).

Conclusions: Here we report a mechanism by which talc enhances the pro-oxidant state in normal and ovarian cancer cells through induction of gene point mutations in key oxidant enzymes, altering their activities.

